

We claim:

1. A method for transiently permeabilizing a target cell, comprising the steps of

- 5 (a) illuminating a population of substantially stationary cells contained in a frame;
- (b) obtaining a static representation of at least one property of light directed simultaneously from the frame;
- 10 (c) locating a target cell in the population of cells, wherein the target cell is located with reference to the static representation; and
- (d) irradiating the target cell with a pulse of radiation;

whereby the target cell is transiently permeabilized.

15 2. A method for transiently permeabilizing a target cell, comprising the steps of

- (a) illuminating a population of cells contained in a frame, wherein the cells are illuminated through a lens having a numerical aperture of  
20 at most 0.5;
- (b) detecting at least one property of light directed from the frame and through the lens;
- (c) locating a target cell in the population of cells, wherein the target cell is located with  
25 reference to the detected property of light; and
- (d) irradiating the target cell with a pulse of radiation;

whereby the target cell is transiently permeabilized.

3. A method for transiently permeabilizing a target cell, comprising the steps of

- (a) illuminating a population of cells contained in a frame;
- 5 (b) detecting at least one property of light directed from the frame;
- (c) locating a target cell in the population of cells, wherein the target cell is located with reference to the detected property of light;
- 10 and
- (d) irradiating the target cell with a pulse of radiation, wherein the pulse of radiation has a diameter of at least 5 microns at the point of contact with the target cell;
- 15 whereby the target cell is transiently permeabilized.

4. A method for transiently permeabilizing a target cell, comprising the steps of

- (a) illuminating a population of cells contained in a frame;
- 20 (b) detecting at least one property of light directed from the frame;
- (c) locating a target cell in the population of cells, wherein the target cell is located with reference to the detected property of light;
- 25 and
- (d) irradiating the target cell with a pulse of radiation, wherein the pulse of radiation delivers at most  $1 \mu\text{J}/\mu\text{m}^2$ ;
- whereby the target cell is transiently permeabilized.

5. The method of claim 2, 3 or 4, wherein the population of cells is substantially stationary.

6. The method of claim 2, 3 or 4, wherein the property of light detected in step (b) is obtained as a static representation of light transmitted simultaneously from the frame, whereby the target cell located in step (c) is located with reference to the static representation.

7. The method of claim 2, 3 or 4, wherein step (b) further comprises obtaining a static representation of the at least one property of light transmitted simultaneously from the frame, whereby step (c) further comprises locating the target cell with reference to the static representation.

8. The method of claim 1, 2, 3 or 4, wherein at least one property of light is fluorescence and the target cell is located with reference to the fluorescence.

9. The method of claim 1, 3 or 4, wherein the population of cells is illuminated through a lens having numerical aperture of at most 0.5 and the target cell is located with reference to a property of light directed from the frame and through the lens.

10. The method of claim 2, wherein the lens has a numerical aperture of at most 0.4.

11. The method of claim 2, wherein the lens has a numerical aperture of at most 0.3.

12. The method of claim 2, wherein the lens has flat field correction.

13. The method of claim 2, wherein the lens has a working distance of at least 5 mm.

5           14. The method of claim 2, wherein the lens has a working distance of at least 10 mm.

15. The method of claim 1, 2 or 4, wherein the pulse of radiation has a diameter of at least 5 microns at the point of contact with the target cell.

10           16. The method of claim 3, wherein the pulse of radiation has a diameter of at least 7 microns at the point of contact with the target cell.

15           17. The method of claim 3, wherein the pulse of radiation has a diameter of at least 10 microns at the point of contact with the target cell.

18. The method of claim 3, wherein the pulse of radiation has a diameter of at least 20 microns at the point of contact with the target cell.

20           19. The method of claim 1, 2 or 3, wherein the pulse of radiation delivers at most  $1 \mu\text{J}/\mu\text{m}^2$ .

20. The method of claim 4, wherein the pulse of radiation delivers at most  $0.1 \mu\text{J}/\mu\text{m}^2$ .

21. The method of claim 4, wherein the pulse of radiation delivers at most  $0.01 \mu\text{J}/\mu\text{m}^2$ .

22. The method of claim 1, further comprising the step of (e) adjusting the direction of the pulse of radiation to irradiate a second target cell in the population, whereby the second target cell is transiently permeabilized.

23. The method of claim 1, wherein the frame has an area of at least 50 mm<sup>2</sup>.

24. The method of claim 1, wherein the frame has an area of at least 85 mm<sup>2</sup>.

25. The method of claim 1, wherein the frame has an area of at least 115 mm<sup>2</sup>.

26. The method of claim 1, wherein the population of cells is illuminated with a laser.

27. The method of claim 1, wherein the population of cells is illuminated with a lamp.

28. The method of claim 1, wherein the population of cells is illuminated with light selected from the group consisting of visible, ultraviolet, and infrared wavelengths.

29. The method of claim 1, wherein the property of light is selected from the group consisting of visible, ultraviolet, or infrared wavelengths.

30. The method of claim 1, wherein the property of light is transmittance and the target cell is located with reference to the transmittance.

31. The method of claim 1, wherein the property of light is polarization and the target cell is located with reference to the polarization.

32. The method of claim 1, wherein the  
5 property of light is reflectance and the target cell is located with reference to the reflectance.

33. The method of claim 1, wherein the property of light is phase contrast illumination and the target cell is located with reference to the phase  
10 contrast illumination.

34. The method of claim 1, wherein the property of light is intensity and the target cell is located with reference to the intensity.

35. The method of claim 1, wherein greater  
15 than 50% of the irradiated target cells are viable after the method is performed.

36. The method of claim 1, wherein greater than 80% of the irradiated target cells are viable after the method is performed.

20 37. The method of claim 1, wherein greater than 90% of the irradiated target cells are viable after the method is performed.

38. The method of claim 1, wherein the target cell is a procaryotic cell.

39. The method of claim 1, wherein the target cell is a eucaryotic cell.

40. The method of claim 1, wherein the target cell is selected from the group consisting of an animal  
5 cell, plant cell, yeast cell, human cell and non-human primate cell.

41. The method of claim 1, wherein the population of cells contains cells associated with an exogenous label.

10 42. The method of claim 41, wherein the label is a fluorophore.

43. The method of claim 1, wherein the target cell is associated with an exogenous label.

44. The method of claim 1, wherein the target  
15 cell is in the presence of an exogenous molecule, whereby the exogenous molecule enters the transiently permeabilized cell.

45. The method of claim 44, wherein the exogenous molecule is selected from the group consisting  
20 of a nucleic acid, polypeptide, carbohydrate, lipid, and small molecule.

46. The method of claim 45, wherein the small molecule is a dye capable of absorbing visible, ultraviolet or infrared light.

47. The method of claim 44, wherein the molecular weight of the exogenous molecule is greater than 0.1 kiloDalton.

48. The method of claim 44, wherein the  
5 molecular weight of the exogenous molecule is greater than 0.3 kiloDalton.

49. The method of claim 44, wherein the molecular weight of the exogenous molecule is greater than 1 kiloDalton.

10 50. The method of claim 44, wherein the molecular weight of the exogenous molecule is greater than 3 kiloDaltons.

51. The method of claim 44, wherein the molecular weight of the exogenous molecule is greater  
15 than 10 kiloDaltons.

52. The method of claim 44, wherein the molecular weight of the exogenous molecule is greater than 30 kiloDaltons.

53. The method of claim 44, wherein the  
20 molecular weight of the exogenous molecule is greater than 70 kiloDaltons.

54. The method of claim 1, wherein step (b) further comprises obtaining a second static representation of at least one property of light directed  
25 simultaneously from the frame.



55. The method of claim 54, wherein the target cell is located with reference to said first and second static representation.

56. The method of claim 1, wherein steps (c) and (d) are repeated so that more than one target cell is located and irradiated.

57. The method of claim 1, further comprising the steps of

- (e) illuminating a population of cells contained in a second frame
- (f) obtaining a static representation of at least one property of light directed from the second frame and through the lens, and repeating steps (c) through (d).

58. The method of claim 57, wherein the population of cells remains in a substantially stationary location relative to the lens.

59. The method of claim 57, wherein at least 10,000 cells are irradiated per minute.

60. The method of claim 57, wherein at least 20,000 cells are irradiated per minute.

61. The method of claim 57, wherein at least 50,000 cells are irradiated per minute.

62. The method of claim 57, wherein at least 100,000 cells are irradiated per minute.

63. The method of claim 57, further comprising the step of

(g) moving the population of cells relative to the lens and repeating steps (a) through (f).

5           64. The method of claim 57, wherein steps (a) through (f) are automated.

65. The method of claim 1, further comprising the steps of

10           (e) moving the population of cells relative to the lens and repeating steps (a) through (d).

66. The method of claim 1, wherein steps (a) through (d) are automated.

67. The method of claim 1, wherein the static representation comprises an image.

15           68. The method of claim 1, wherein the static representation comprises a set of data stored in computer memory.

69. The method of claim 1, further comprising a camera having a magnification between 2X and 40X.

20           70. The method of claim 1, further comprising a camera having a magnification between 2.5X and 25X.